

Parvovirus B19 Infection Localized in the Intestinal Mucosa and Associated with Severe Inflammatory Bowel Disease[▽]

Loris Pironi,¹ Francesca Bonvicini,² Paolo Gionchetti,³ Antonia D'Errico,⁴ Fernando Rizzello,³ Catia Corsini,¹ Laura Foroni,⁵ and Giorgio Gallinella^{2*}

Center for Chronic Intestinal Failure, Department of Internal Medicine and Gastroenterology,¹ Division of Microbiology, Department of Clinical and Experimental Medicine,² Center for Inflammatory Bowel Disease, Department of Internal Medicine and Gastroenterology,³ Division of Pathology, Department of Oncology and Hematology,⁴ and Division of Vascular Surgery, Department of Specialistic Surgical and Anesthesiological Sciences,⁵ University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy

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Infection by human parvovirus B19 is widespread and can be associated with a wide range of different pathologies and clinical manifestations. We provide the first evidence of localization of an active parvovirus B19 infection in the intestinal mucosa and its association with a severe inflammatory bowel disease, characterized by duodenal villous atrophy with increased intraepithelial lymphocytes and inflammatory infiltrates in the colonic mucosa. Virus in the intestinal mucosa was detected in cells of the inflammatory infiltrate, identified as T lymphocytes and selectively localized in sites of active tissue degeneration.

CASE REPORT

In February 2004, a diagnosis of extensive ulcerative colitis in a 52-year-old Caucasian man was made. Treatment with oral methylprednisolone (20 to 60 mg/day) resulted in improvement of the condition.

In June 2004, he presented with a subacute bulbar syndrome. Spinal fluid analysis showed increased white blood cells. No specific infection was detected, and, as neoplastic and autoimmune diseases were excluded, an unrecognized infective etiology was hypothesized. Treatment with acyclovir was begun, along with corticosteroid maintenance (methylprednisolone, 20 to 60 mg/day). Encephalitis healed within 2 months.

In August 2004, ulcerative colitis was not responsive to medical treatment (methylprednisolone, 40 to 60 mg/day). A total colectomy with temporary ileostomy was performed, and corticosteroid treatment was stopped.

In January 2005, bloody diarrhea with an ileostomy output of 5,000 to 6,000 ml/day, severe malabsorption, and systemic inflammatory reaction appeared. Ileoscopy revealed a diffuse superficial ulceration of the ileum. Histology showed a reactive inflammatory infiltrate with eosinophils as well as an increased number of intraepithelial lymphocytes associated with degenerative changes of the epithelial lining. A diagnosis of aspecific enteritis was made. Treatment with intravenous methylprednisolone (60 mg/day), oral fasting, and total parenteral nutrition were started.

In March 2005, histology of the ileum showed ulcerative enteritis with focal pseudomembranes. Treatment with oral azathioprine, 50 to 100 mg/day, was started.

In May 2005, ileostomy output was 3,000 to 4,000 ml/day and histology of the ileum was unchanged. Upper endoscopy

showed a granular duodenal mucosa with severe villous atrophy, crypt hyperplasia, and inflammatory infiltrate with plasma cells, and more than 25 intraepithelial lymphocytes per 100 enterocytes were observed. Autoimmune enteropathy was excluded by a search for serum enterocyte autoantibodies, and intestinal lymphoma was excluded by immunohistochemistry. Serum chromogranin A concentration was normal. Tests for antitransglutaminase and antigliadin antibodies were negative. Serum immunoglobulin A (IgA) concentration was normal. HLA typing for celiac disease indicated that DQA1*0501 was absent, DQB1*0201 was present, and DQB1*0302 was absent; that for chronic inflammatory bowel disease indicated that DRB1*07 was present.

Infections by *Salmonella*, *Brucella*, *Shigella*, *Campylobacter*, *Yersinia*, *Mycobacterium*, *Trypanosoma*, *Toxoplasma*, *Aspergillus*, or *Cryptococcus* spp. or by *Treponema pallidum*, *Borrelia burgdorferi*, *Giardia intestinalis*, *Entamoeba histolytica*, *Schistosoma mansoni*, adenovirus, rotavirus, enterovirus, human immunodeficiency virus, herpes simplex virus type 1 or 2, varicella-zoster virus, Epstein-Barr virus, or cytomegalovirus were excluded by fecal and duodenal cultures, serum and fecal antibody analysis, and serum and ileal mucosa PCR analysis for virus DNA and RNA.

Having found no pathogenetic cause, the search for a viral infection was enlarged, and unexpected evidence for a parvovirus B19 infection was obtained (7). Results of laboratory tests for the detection of B19 virus nucleic acids and specific antibodies in serum samples through the course of the disease are shown in Table 1. In the first available serum sample (May 2005), B19 virus DNA was present at 1.00×10^4 IU/ml, concomitantly with the presence of low-level anti-B19-specific IgG antibodies. Measurement of VP1-IgG avidity and epitope-type-specific (ETS) reactivity (performed at the Institute of Virology, Haartman Institutes, Helsinki, Finland) showed both high IgG avidities (41.5%; cutoff for high avidity, 25%) and ETS ratios (24.0; cutoff index for past infection, 10.0), typical

* Corresponding author. Mailing address: Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, Via Massarenti, 9, 40138 Bologna, Italy. Phone: 39 051 4290930. Fax: 39 051 307397. E-mail: giorgio.gallinella@unibo.it.

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TABLE 1. Detection of B19 virus and of specific antibodies in biopsy and serum samples^a

Date (day/mo/yr)	Intestinal mucosa	Result ^b for:			Serum or bone marrow	qPCR result ^f (IU/ml)	Presence ^g of:	
		PCR ^c	ISH ^d	IHC ^e			IgM	IgG
05/08/2004	Colon	Pos	Pos	Pos				
25/01/2005	Ileum	Neg	ND	ND				
02/03/2005	Ileum	Pos	ND	ND				
05/05/2005	Ileum	Neg	ND	ND	Serum	1.00×10^4	0.9	2.3
31/05/2005					Serum	7.37×10^4	0.2	5.9
26/09/2005	Ileum	Pos	ND	ND	Serum	ND	0.6	3.5
12/10/2005	Ileum	ND	Pos	ND				
	Duodenum	ND	Pos	ND				
20/10/2005	Ileum	Pos	Pos	ND				
18/01/2006					Bone marrow	1.93×10^5	ND	ND
19/01/2006	Ileum	ND	Pos	ND	Serum	3.08×10^4	0.2	4.5
21/03/2006	Ileum	ND	Neg	ND	Serum	1.10×10^2	0.3	3.8
17/06/2006	Ileum	ND	Neg	Neg	Serum	$<1.00 \times 10^2$	0.1	3.7
	Duodenum	ND	Pos	Pos	Serum	$<1.00 \times 10^2$	0.1	3.7
21/07/2006	Ileum	Neg	Neg	ND	Serum	$<1.00 \times 10^2$	ND	ND
05/10/2006					Serum	$<1.00 \times 10^2$	0.3	2.0
16/01/2007	Ileum	Neg	Neg	ND	Serum	$<1.00 \times 10^2$	0.6	5.3

^a ND, not done.^b Pos, positive; Neg, negative.^c Presence of B19 virus DNA, as determined by a standardized PCR–enzyme-linked immunosorbent assay (14) following nucleic acid extraction from formalin-fixed, paraffin-embedded tissue sections.^d Viral DNA detected by both a digoxigenin-labeled DNA probe and a biotin-labeled peptide nucleic acid probe, followed by immunochemical detection and chromogenic substrate development (2). ISH, in situ hybridization.^e Viral VP proteins detected by anti-VP1/2 monoclonal antibody (Chemicon; 8293), followed by alkaline phosphatase-conjugated anti-mouse secondary antibody and chromogenic substrate development (15). IHC, immunohistochemistry.^f Presence of B19 virus DNA determined and quantitated by a calibrated real-time quantitative PCR (qPCR) assay; values are expressed by using the international standard NIBSC 99/800 as the reference sample (6). The detection threshold of the assay is 1.00×10^2 IU/ml.^g Presence of specific anti-B19 IgM and IgG, as determined by a commercial enzyme immunoassay (Biotrin); results are expressed as optical density/coefficient of variation values, as recommended by the manufacturer; values below 0.9 should be considered negative and those above 1.1 positive.

of mature immunity (4) and compatible with a primary infection associated with earlier phases of the disease.

Retrospective investigation was performed on the available biopsy samples (Table 1). In situ hybridization and immunohistochemical analysis revealed the presence of viral nucleic acids and capsid proteins in cells of the mucosal inflammatory infiltrate of the ileum (Fig. 1A) and of the colon at the time of the colectomy (Fig. 1B), identified as T lymphocytes on the basis of histological characteristics and tissue distribution. Double-labeling immunofluorescence microscopy analysis per-

formed on the colon biopsy samples positively assessed the presence of intraepithelial CD3-positive lymphocytes expressing B19 virus capsid proteins in necrotic colonic crypts and in the adjacent lamina propria (Fig. 2A) and their absence in crypts still retaining a normal architecture (Fig. 2B). CD3 lymphocytes diffusely present in the stroma and CD20 lymphocytes organized into lymphoid follicles did not show expression of B19 virus capsid proteins.

Treatment with intravenous immunoglobulins (25 g/day for 10 days in May, June, and August 2005) was performed, fol-

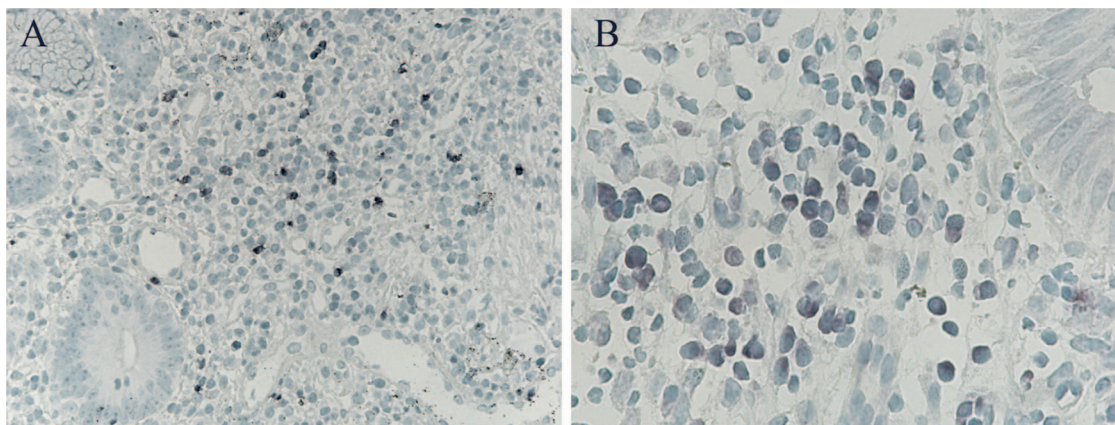


FIG. 1. In situ hybridization analysis for detection of B19 virus nucleic acids on biopsy samples. (A) Ileum showing aspects of aspecific enteritis. (B) Colon showing aspects of ulcerative colitis. Viral DNA was detected by a virus-specific, biotin-labeled peptide nucleic acid probe, followed by immunochemical detection and chromogenic substrate development (2). Infected cells are evident (purple stained) and have been identified as T lymphocytes in the context of an inflammatory infiltrate.

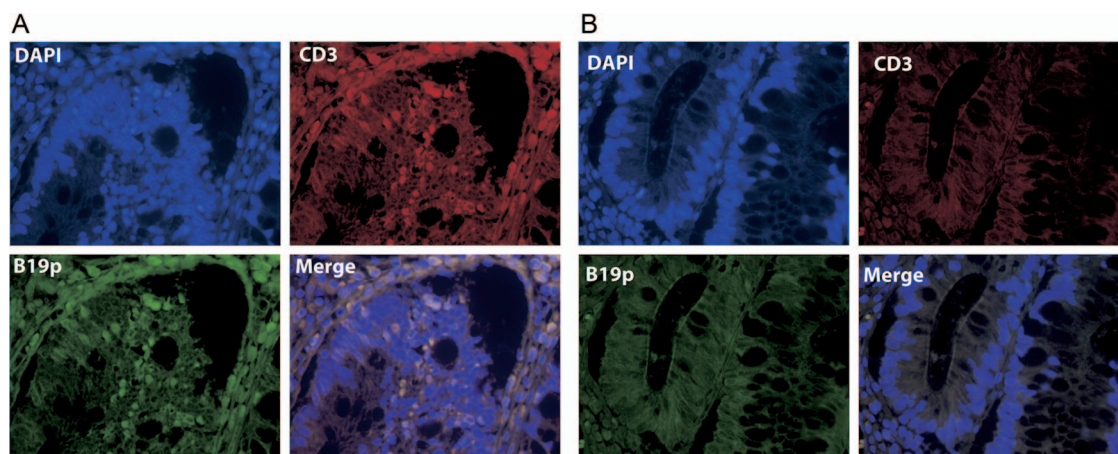


FIG. 2. Double immunofluorescence (IF) of colon histological sections. The sections were dewaxed with xylene and rehydrated through decreasing concentrations of ethanol; tissue antigenicity was recovered using a heat-based retrieval. After unspecific binding sites were blocked with a solution containing 2% swine serum, 2% goat serum, and 1% bovine serum albumin, slides were incubated overnight at 4°C with a mixture of the primary antibodies (anti-B19V VP2 polyclonal rabbit antibody, diluted 1:200, and monoclonal mouse anti-human CD3 clone F7.2.38, diluted 1:40; DakoCytomation) and then in a mixture of fluorescence-conjugated secondary antibodies (fluorescein isothiocyanate polyclonal swine anti-rabbit, diluted 1:200 [DakoCytomation], and rhodamine-conjugated goat affinity-purified antibody to mouse IgG, diluted 1:200 [MP Bio-medicals]). Finally, samples were mounted and the nuclei were counterstained with DAPI (4',6-diamidino-2-phenylindole) using the Prolong antifade reagent kit (Molecular Probes). Negative controls were performed by omitting the primary antibodies. In preliminary experiments, each primary antibody was also tested individually to exclude unspecific cross-reactions with secondary antibodies or optical spillover between green and red signals (data not shown). Microscopy analysis was performed with a DMI6000 B inverted fluorescence microscope (Leica Microsystems SpA, Milan, Italy). Staining with CD3 monoclonal antibody yielded red fluorescence, and staining with anti-B19 VP2 protein (B19p) polyclonal antibody yielded green fluorescence. Cell nuclei were labeled blue with DAPI. Double-stained cells are yellow in the merged image (15). Original IF magnification, $\times 40$. (A) Intraepithelial CD3⁺ T lymphocytes present in a necrotic colonic crypt are infected by B19 virus as shown by expression of viral capsid proteins. Infected CD3 cells are also present in the adjacent stroma. Epithelial cells are detached from the gland basal lamina and show degenerative aspects. (B) On the same tissue section, an adjacent colon crypt shows a normal architecture. Intraepithelial CD3 lymphocytes are not observed, and B19 VP proteins are not expressed. No degenerative aspects are present, and the lamina propria is normal.

lowed by decreases in the peripheral blood viral load, which were observed following each immunoglobulin administration. A gluten-free diet was started, associated with a slow tapering off of methylprednisolone, reaching 20 mg/day in September 2005. Oral azathioprine, 100 mg/day, was maintained.

The presence of B19 virus in the duodenal and ileal mucosa was confirmed in September and October 2005 and in January 2006. B19 virus DNA was also detected by PCR in a bone marrow aspirate in January 2006 (Table 1).

In March 2006, ileal histology showed atrophy and distortion of the villi associated with aspects of chronic inflammation and fibrosis of the lamina propria. B19 virus was not detected in the mucosa, while viremia was at the minimal detectable levels. Oral azathioprine was stopped, while oral methylprednisolone was maintained at 20 mg/day.

In June 2006, ileostomy output was 600 to 800 ml/day. Both the ileum and duodenum histology showed an improvement in the mucosal inflammatory infiltrate and villous atrophy. B19 virus nucleic acids and capsid proteins were still present in the duodenal but not in the ileal mucosal T lymphocytes. Peripheral blood viral load decreased below the detection threshold. Parenteral nutrition was stopped, oral gluten challenge was started, and oral methylprednisolone was maintained at 20 mg/day.

In July 2006, ileal histology showed only mild inflammatory infiltrate with mild villous and crypt atrophy. The virus was no longer detected in the intestinal mucosa and peripheral blood. In October 2006 and January 2007, the ileostomy output remained 600 to 800 ml/day, the mucosal inflamma-

tory infiltrate was minimal, and the villous and crypt morphology was normal.

Infection by human parvovirus B19 is widespread and can be associated with a wide range of clinical manifestations, whose characteristics and outcome mainly depend on the physiological and immune status of the infected individual (20). The virus shows a marked tropism for erythroid progenitor cells in the bone marrow, exerting a cytotoxic effect and causing a block in erythropoiesis. In a compensated and immunocompetent host this block can be unapparent and is usually relieved by the development of a neutralizing immune response; otherwise, it may become apparent as transient or persistent erythroid aplasia in subjects with underlying stressed erythropoiesis or impaired antiviral immune response. Common manifestations of infection are erythema infectiosum in children and postinfection arthropathies mainly affecting adults; furthermore, the virus has been implicated in a growing spectrum of other different pathologies, among them vasculitis, myocarditis, encephalitis, and autoimmune connective tissue diseases.

In our patient, a persistent infection was documented by the detection of B19 virus in the peripheral blood for a prolonged period of time, despite the presence of specific antiviral antibodies. In such context, we present the unexpected evidence of localization of productively infected T lymphocytes in the intestinal mucosa and the association of the infection with a

severe inflammatory bowel disease. Sequencing of amplified genomic segments obtained from serum, bone marrow, and biopsy samples yielded a standard genotype 1 sequence, also showing sequence identity in the different samples and conservation all through the course of the persistent infection. Thus, it is unlikely that the observed infection of T lymphocytes might be linked to the emergence of a variant genotype with altered cellular tropism, although such a possibility cannot be excluded. The major issue is then whether in this case B19 virus should be considered an active agent of inflammation in the observed bowel disease or as a mere bystander in otherwise inflamed tissue.

Although aspecific intestinal symptoms in association with acute B19 virus infection are commonly described, the presence of the virus in the intestinal mucosa has not been previously reported in the literature. In our own experience, including analysis of a series of biopsy samples from 12 cases of ulcerative colitis (our unpublished data), this is the first case showing the presence of B19 virus in the intestinal mucosa. As B19 virus was detected at relatively higher levels in the bone marrow, which is assumed to be the major site of viral persistence, we cannot exclude the possibility that the virus could be aspecifically disseminated throughout the body and that infected lymphocytes nonspecifically trafficked to sites of inflammation and other tissues as well. However, in the present case, the course of disease appeared markedly characterized by the association of B19 virus infection with the severe bowel inflammation.

In fact, clinical, pathological, and virological data suggest an active role for B19 virus infection in the development and evolution of inflammatory bowel disease in a genetically predisposed host. Infective disorders of the gastrointestinal tract may have histological features that mimic Crohn's disease and ulcerative colitis (11) or that can be associated with intestinal villous atrophy, with or without increased intraepithelial lymphocytes, which may require a differential diagnosis of a refractory celiac disease (3). In the present case, histological features associated with B19 virus infection were at first typical of ulcerative colitis and then, in the small bowel, were characterized by villous atrophy with increased intraepithelial lymphocytes and a reactive inflammatory infiltrate of the mucosa. On the other hand, an etiological role for B19 virus in the encephalitis (bulbar syndrome) observed in the initial phase of the disease cannot be directly assessed because of the lack of virological testing but can be hypothesized because of the temporal coincidence and clinical evolution (1). The observation that our patient was positive for HLA class II antigen alleles DRB1*07 and DQB1*02 agrees with previous findings which suggest a role for patient genotype in clinical manifestation of B19 virus infection. The frequencies of the HLA DRB1*01, DRB1*04, and DRB1*07 alleles were found to be significantly higher in patients with symptomatic acute B19 virus infection than in control subjects (10), especially in those patients with meningoencephalitis (9). A case of ulcerative colitis which developed 8 years after human B19 virus-related encephalopathy has been described previously, and the authors hypothesized that an HLA DRB1-associated congenital T-cell dysfunction would have allowed the occurrence of both diseases in the same patient (19). It seems of interest that DRB1*07 has been reported to be associated (usually with DQB1*02) with a va-

riety of diseases and syndromes, including celiac disease and Crohn's disease (8, 16, 17), whose histological features are similar to those observed in our patient. Corticosteroid and azathioprine treatment may have conditioned the course of infection, causing an impairment of the immune response and its consequent inability to control viral replication and clear viral infection. Suspension of corticosteroids after the colectomy was mainly associated with exacerbation of bowel inflammatory manifestations, while intravenous immunoglobulins were probably effective in the resolution of the infection during the late evolution of the disease.

An active role for B19 virus in the development of inflammatory bowel disease can also be supported by the virological findings in both the colon and ileal mucosa. In fact, besides viral genomes, which were detected by PCR in biopsy samples, productively infected cells were detected by *in situ* hybridization and immunohistochemistry. The presence of viral genomes, detected only by means of PCR, is common in a variety of tissues and cannot be considered a sign of active infection, while detection of viral nucleic acids and capsid antigens in cells is direct evidence of productive infection and indicates the extent of cellular and tissue distribution of the virus. It is worth noting that virus in the intestinal mucosa was not detected in epithelial cells; rather, it was detected in cells of the inflammatory infiltrate of the mucosa, identified as CD3 T lymphocytes, and selectively localized in sites of active tissue degeneration.

Although not classically considered target cells for B19 virus, lymphocytes can express Ku80, recently identified as a possible receptor for B19 virus (13). B19 virus-infected T lymphocytes have previously been identified in the synovial tissues of patients with rheumatoid arthritis (18). In our case, a possible pathogenetic mechanism may involve persistence of infection in the bone marrow and Ku80-mediated infection of T lymphocytes; expression of viral nonstructural protein NS can transactivate expression of interleukin-6 and tumor necrosis factor alpha (5, 12), so that infected cells may become activated and sustain an inflammatory response exceptionally targeted to the bowel mucosa.

In conclusion, our findings suggest both a novel cellular target and pathogenetic potential of a widespread human virus and a new potential mechanism of human inflammatory bowel disease in genetically susceptible individuals.

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